Journal of Biochemical and Biophysical Methods, 12 (1986) 29–36
Elsevier

BBM 00508

Isolation and fractionation of total nucleic acids from tissues and cells

Stephen A. Krawetz, J. Christopher States * and Gordon H. Dixon

Department of Medical Biochemistry, Health Sciences Centre, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1

(Received 18 June 1985)
(Accepted 15 July 1985)

Summary

A simple and efficient method was devised to permit the isolation of total nucleic acids (poly(A$^+$) and poly(A$^-$) RNAs and DNA) from cells and tissues (e.g. testis) enriched in DNA. Chaotropic reagents (guanidine thiocyanate and LiBr) were utilized to inactivate nucleases rapidly, minimize the viscosity of the homogenization solution and to precipitate RNA selectively (LiBr). Both total and poly(A$^+$)-enriched mRNAs were recovered in a biologically active form as demonstrated by their ability to programme an in vitro translation reaction. High molecular weight DNA (> 22 kilobases) was recovered from the LiBr-soluble supernatants by selective ethanol precipitation, subsequently purified and was in a form suitable for further biochemical analysis.

Key words: mRNA; DNA; RNA; nucleic acid isolation; chaotropic reagents; tissues and cells.

Introduction

Chaotropic reagents are very successful in the rapid and complete denaturation of RNase [1]. This has permitted the isolation of biologically active messenger RNAs (mRNAs) from tissues rich in RNase [2] and for the isolation of large mRNAs [3–5]. Numerous procedural anecdotes have been introduced, which in general, have optimized the isolation procedure for a specific tissue [6–8]. These various procedures are not necessarily optimized for all tissues, although they are generally successful in isolating some biologically active mRNA. With the exception of one of the original reviews by Cox [9] the opportunity to isolate the DNA simultaneously with the RNA has largely been ignored. In this communication we describe our

* *Present address*: IDR-720, Children's Hospital Research Foundation, Elland and Bethesda Avenues, Cincinnati, OH 45220, U.S.A.

studies on the optimization of the simultaneous isolation of biologically active mRNA and DNA from testis and cell cultures.

Materials and Methods

Guanidine-HCl (GHCl) was purchased from Bethesda Research Laboratories and guanidine thiocyanate (GSCN) from EM Science. *N*-lauroyl sarcosine (sodium salt) and dithiothreitol (DTT) were purchased from Sigma Chemical Company. [^{35}S]Methionine was purchased from Amersham. Mature bull testes were a generous gift from Dr. R.B. Church and the Church Ranch (Calgary, Alberta) and bovine liver was a generous gift from Dvorkin Meat Packers (Calgary, Alberta). All other chemicals were purchased from sources previously described [5].

Solutions employed in the isolation and translation of RNA were prepared with autoclaved water.

Bull testes and liver were quickly dissected and immediately frozen and stored in liquid N_2.

Isolation of nucleic acids

Isolation of RNA from liver with GHCl

RNA was isolated from fresh bull liver by the GHCl/*N*-lauroyl sarcosine extraction method as previously described [6].

Isolation of RNA from testis with GSCN and LiBr

Fresh bull testis (50 g) was powdered in liquid nitrogen and 5 vol (w/v) of homogenization solution (4 M guanidine thiocyanate, 2% 2-mercaptoethanol (w/v) and 0.1 M Tris-HCl, pH 7.2) was added. The resulting suspension was immediately mixed with a Teckmar homogenizer for 1 min at full speed. *N*-lauroyl sarcosine was added to the homogenized suspension to a final concentration of 0.5% (w/v) and dissolved with a 10-s Teckmar homogenizer pulse. Insoluble material was then pelleted by centrifugation with the use of a Sorvall GSA rotor at 10 000 rpm for 10 min at 5°C and the supernatant removed. To the supernatant, solid LiBr was added slowly with constant mixing to a final concentration of 2 M and 0.025 vol of 1 N acetic acid was also added. RNA was precipitated overnight from the 2 M LiBr solution at 5°C. The precipitated RNA was pelleted by centrifugation with the use of a Sorvall HGL4 rotor at 5000 rpm for 90 min at 5°C, the DNA-containing supernatant was removed and stored separately at 5°C. The RNA pellet was redissolved in 1 vol (with respect to the original tissue weight) of resuspension solution (6 M GHCl, 10 mM DTT, 20 mM EDTA and 0.1 M Tris-HCl, pH 7.2) with the aid of a 30-s Teckmar homogenizer pulse. The RNA was precipitated overnight at −20°C following the addition of 0.06 vol of 3 M NaAc, pH 5.2 and 0.5 vol of absolute ethanol. The RNA was then pelleted at 9000 rpm for 30 min at −5°C in a Sorvall HB4 rotor and the supernatant was removed. The RNA pellet was redissolved in 10 mM Tris-HCl buffer, pH 7.4 containing 2 mM DTT, 4 mM EDTA and

Corrigendum

Krawetz, S.A., States, J.C. and Dixon, G.H.: Isolation and fractionation of total nucleic acids from tissues and cells. J. Biochem. Biophys. Methods 12, 29–36 (1986).

Please substitute lines 7 and 8 on page 31 with those given here:

HeLa cells (4 dishes) were lysed at 80% confluency in 2 ml per 100 mM dish of the homogenization solution (see above) containing 2 M LiBr, 0.5% *N*-lauroyl sarcosine and 10 mM EDTA. The resulting suspen- ....

0.5% sodium dodecyl sulfate (SDS) with the aid of short (10-s) Teckmar homogenizer pulses and heating at 60°C. The solution was subsequently quick chilled. NaCl was added to a final concentration of 0.5 M and insoluble material was removed by centrifugation. The resulting solution was chromatographed on oligo (dT)-cellulose as described [6].

Isolation of RNA from cell culture with GSCN and LiBr

HeLa cells (4 dishes) were lysed at 80% confluency in 2 ml per 100 mM dish of the homogenization solution (see above) containing 2 M LiBr. The resulting suspension was transferred to a corex tube and 0.025 vol of 1 N acetic acid and 0.5 vol of absolute ethanol were added. The resulting solution was mixed by inversion and RNA was precipitated overnight at −20°C. It should be noted that this solution may freeze. In this case, immediately prior to the recovery of the RNA, the frozen solid is quickly melted at room temperature. The precipitated RNA was pelleted by centrifugation at 10 000 rpm for 15 min at −10°C in a Sorvall HB4 rotor. The DNA-containing supernatant was removed and stored separately at 5°C. The RNA-containing pellet was redissolved at 1 ml per dish of resuspension solution (see above). This was aided by extensive vortexing. Insoluble material was removed by centrifugation and the soluble RNA was precipitated overnight at −20°C following the addition of 0.06 vol of 3 M NaAc, pH 5.2 and 0.5 vol of absolute ethanol. The RNA was again pelleted as in the above, dissolved in a minimum volume of 0.3 M NaAc, pH 5.2, and precipitated overnight at −20°C following the addition of 2.5 vol of absolute ethanol. The RNA was then pelleted as described in the above and subsequently washed with absolute ethanol. The RNA was then dried with the use of a speed-vac concentrator and dissolved in a convenient volume of water.

Isolation of DNA from guanidine supernatants

DNA was recovered from the DNA-containing supernatant by the addition of another 0.5 vol of absolute ethanol (a total of 1 vol). DNA was further purified by RNase A digestion prior to proteinase-K treatment and phenol-chloroform extraction as described [10].

DNA was also prepared from nuclei isolated as previously described [10]. Nuclei were lysed in homogenization buffer (see above) and the DNA was recovered as a spool on a pasteur pipette by the addition of 0.06 vol of NaAc, pH 5.2, and the layering of absolute ethanol. DNA was further purified by RNase A digestion prior to proteinase-K treatment and phenol-chloroform extraction as described [10].

Translation of mRNA and analysis of the translated products

mRNA-dependent gel-filtered rabbit reticulocyte lysate was prepared and utilized as previously described [5]. Translated proteins were resolved on a 10% polyacrylamide-SDS slab gel [11] and products visualized by fluorography [12].

Electrophoresis of nucleic acid isolates

DNA samples were resolved on a 0.7% agarose gel as previously described [10] and RNA samples on a methyl mercury-containing agarose gel [13].

Results and Discussion

In our numerous attempts to isolate RNA from bull testis with the use of the methods that were successful for the isolation of large quantities of RNA from aorta [6], we were not very successful. In general, we experienced similar problems as others, including plugging of the CsCl cushions by DNA preventing the RNA from being recovered in a quantitative manner [7] and a very viscous initial homogenate [2,7,14]. This prevented the complete ethanol precipitation of RNA free of DNA. To circumvent these problems we initially tried to shear the DNA by forcing the homogenized solution through a 26-gauge needle [10]. As shown in Table 1, when testis RNA was prepared by the GSCN isolation method, it was not recovered in the optimum yield.

To circumvent these problems we made use of the property of preferential solubility of DNA in high salt solutions. In order to take full advantage of this and to increase the denaturing capacity of the guanidine thiocyanate homogenization solution a second chaotropic reagent, LiBr (both the anion and cation are chaotropic) was included. The LiBr concentration was systematically varied from 0.5 to 4 M. No additional benefit, i.e. more RNA recovered, was found above a final concentration of 2 M (unpublished results). As shown in Table 1, with the use of the GSCN and LiBr isolation method the yield of both total RNA and poly(A^+) markedly increased as compared to the GSCN isolation method. The GSCN and LiBr isolation method was also very successful for the isolation of RNA from cell cultures (Table 1). It should be noted that in this case since the overall concentration of RNA was much less than that for testis the RNA could only be selectively precipitated with the addition of 0.5 vol of ethanol. This method of precipitation should also be very useful for tissues containing relatively little RNA or for small quantities of tissue (2–5 g) where LiBr precipitation may not be quantitative.

As shown in Fig. 1, all RNA preparations were of similar integrity and high molecular weight RNAs, i.e. 28S, were intact. It should be noted that when ethanol was not utilized to precipitate the RNA (testis) some of the lower molecular weight RNA species (i.e. less than 5.8S), were not recovered, although, as shown in the

TABLE 1

COMPARISON OF THE RECOVERY OF RNA WITH THE USE OF THE VARIOUS EXTRACTION METHODS

Source	Isolation method	Yield (A_{260}/g tissue)		
		Total	poly(A^+)	% poly(A^+)
Bull testis	GSCN [10]	5.09	0.21	4
Bull testis	GSCN and LiBr	23.09	1.49	6
Bull liver	GHCl	39.20	1.60	4
		(A_{260}/100 mM plate)		
Cell culture	GSCN and LiBr	9.00	not determined	

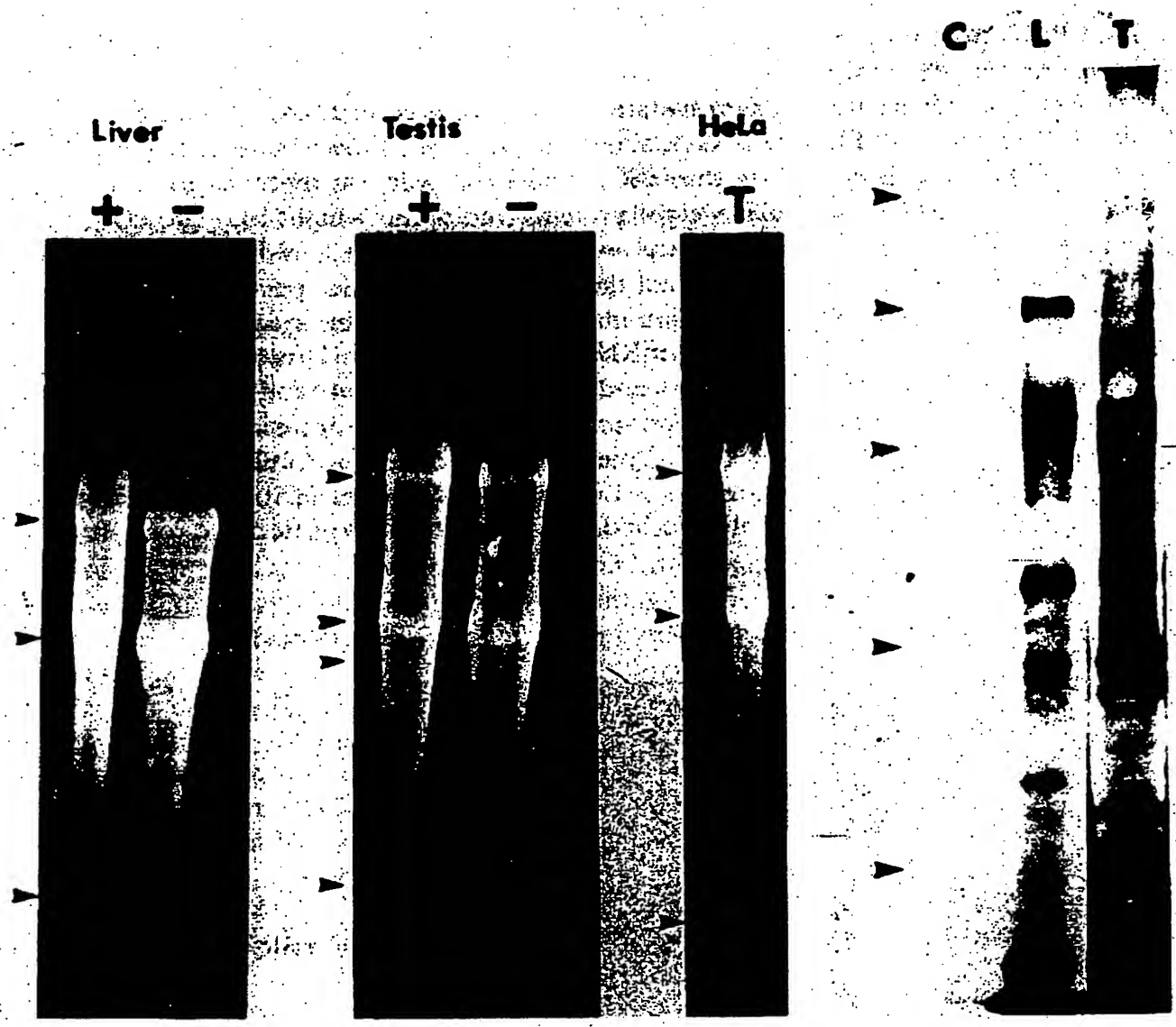

Fig. 1. Methyl mercury agarose gel analysis of the RNAs prepared from tissues and cells. RNA was isolated with the use of chaotropic reagents as described (see Methods). Bull liver and testis RNAs were fractionated by oligio-(dT) cellulose chromatography into poly(A^+) enriched (+) and depleted (−) fractions prior to electrophoresis on a 1% agarose gel. Total (T) HeLa cell RNA was resolved on a 1.5% agarose gel. The liver (+) and (−) lanes contain 27 μg and 32 μg of RNA, respectively. The testis (+) and (−) lanes contain 30 μg and 28 μg of RNA, respectively. The HeLa lane T contains 26 μg of RNA. The migration of the RNA molecular weight standards is indicated by arrows, from top to bottom 28S, 18S, and 5.8S. The bands were visualized by ethidium fluorescence.

Fig. 2. Fluorograph of the in vitro translated polypeptides synthesized with bull liver and testis poly(A^+) enriched mRNAs. The polypeptides were synthesized in a gel-filtered mRNA dependent rabbit reticulocyte lysate in the presence of 30 μCi [^{35}S]methionine in a final reaction volume of 25 μl. The translated products were analyzed on a 10% polyacrylamide-SDS slab gel. Lane C, control, no mRNA added; lane L, 0.27 μg of liver mRNA added that was isolated by the GHCl/*N*-lauroyl sarcosine extraction procedure; lane T, 0.24 μg of testis mRNA added that was isolated by the GSCN and LiBr isolation method. The molecular weight markers are indicated by the arrowheads. From top to bottom they are: phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase and soybean trypsin inhibitor. The dried gel containing the fluor was exposed for 2 days.

HeLa RNA preparation, they may be recovered by precipitation with ethanol.

To examine the biological activity of the RNA prepared by the GSCN and LiBr isolation method the RNA was translated in a gel-filtered mRNA dependent rabbit reticulocyte lysate and the results are shown in Fig. 2. As shown in lane T, the testis mRNA which was prepared by the GSCN and LiBr isolation method programmed the synthesis of large molecular weight proteins (molecular weight of ~90 000). As expected the liver mRNA which was isolated by the GHCl/*N*-lauroyl sarcosine extraction procedure also programmed the synthesis of a wide spectrum of proteins (lane L). As is clearly evident, there are major differences in the spectrum of proteins synthesized from the corresponding mRNAs of these tissues. This probably reflects differences in tissue-specific messages and levels of certain common messages. HeLa cell total RNA was also capable of programming an in vitro translation reaction as measured by the incorporation of [^{35}S]methionine into TCA-insoluble radioactivity. The specific activity as measured in a 1 μl aliquot of the translation reaction was 2.5×10^6 cpm per μg. Thus both methods of preparation permitted the recovery of

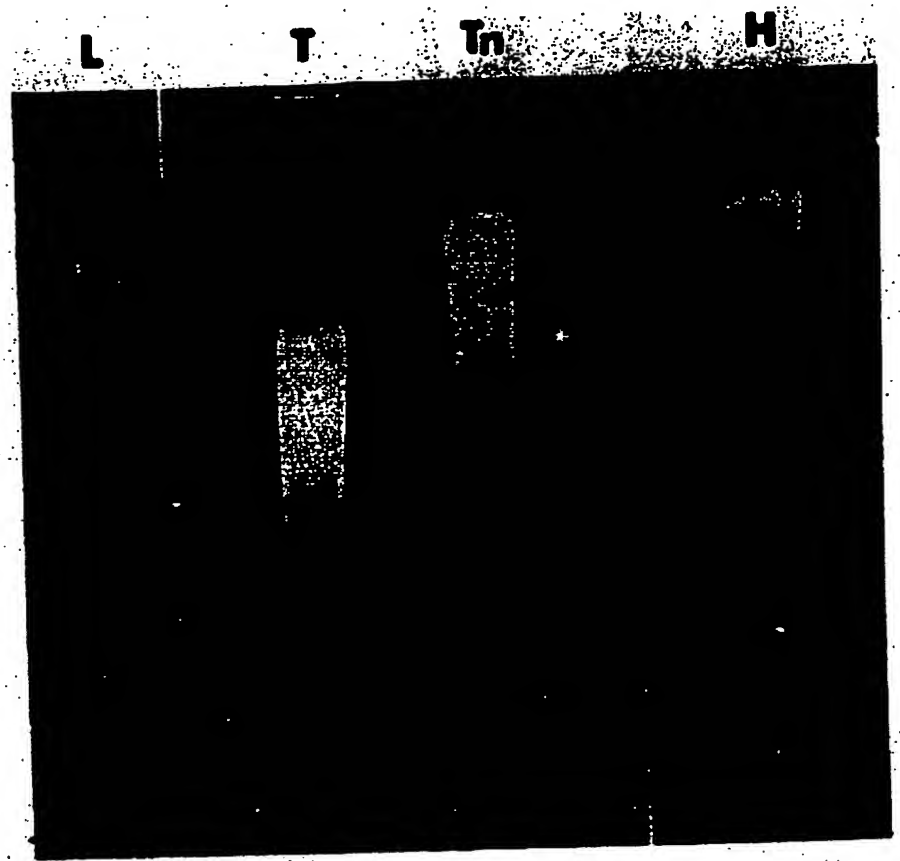

Fig. 3. Comparison of the DNA isolated from the guanidine supernatants. DNA was isolated as described (see Methods), resolved on a 0.7% agarose gel and visualized by ethidium fluorescence. The migration of the DNA isolates is shown. Lane L, lambda Hind III molecular weight standards, from top to bottom 23.1, 9.34, 6.6, 4.4 kb; lane T, 2 μg of bull testis DNA isolated from the GSCN and LiBr supernatant; lane Tn, 2 μg of bull testis DNA isolated from nuclei; and lane H, 2 μg of the HeLa cell DNA isolated from the GSCN and LiBr supernatant.

biologically active mRNA and high molecular weight RNAs.

As originally observed by Cox [9], RNA in chaotropic solutions is selectively precipitated with the addition of 0.5 vol of ethanol and DNA with 1 vol of ethanol. This has permitted the differential isolation of RNA and DNA from the same preparation. As shown in Fig. 3, with the addition of a total of 1 vol of ethanol the DNA can be recovered from the DNA-containing supernatant. There appears to be little if any contaminating low molecular weight species that would correspond to RNA. Furthermore, as evidenced by the relative sizes of the different DNA preparations, depending upon the method of isolation, the integrity of DNA is affected. As shown (lane T) DNA isolated from tissues subjected to extensive shearing forces by homogenization is of relatively small molecular weight. Although this DNA is rather small it has proven suitable for dot blot analysis [10]. This does not imply that high molecular weight DNA cannot be isolated from this tissue. As shown in lane Tn, this was accomplished when nuclei were first isolated and used as the source of DNA.

Isolation of DNA from the DNA-containing supernatants from cell culture nucleic acid isolates offers one main advantage. Since vigorous homogenization is not required to lyse the cells, this precludes the introduction of extensive shearing forces. Thus, the integrity of the DNA is maintained, as evidenced by its high molecular weight (lane H).

In conclusion, the methods described in this communication provide the ability to isolate large quantities of biologically active mRNA from high DNA content tissues. Furthermore, our studies have shown that the DNA can also be isolated from the same preparation in a high molecular weight form suitable for further analysis.

Simplified description of the method and its advantages

A simple procedure is described for the isolation of biologically active poly(A^+) mRNAs, poly(A^-) RNAs and DNA from cells and tissues. The tissue is rapidly homogenized or cells lysed in guanidine thiocyanate and the RNA selectively precipitated from the homogenate with LiBr. DNA is subsequently recovered from the soluble phase by ethanol precipitation and is further purified by RNase A digestion, proteinase K treatment and phenol–chloroform extraction. RNAs are purified from the precipitate by guanidine–HCl extraction and separated into poly(A^+) and poly(A^-) RNAs by oligo(dT)-cellulose chromatography. This method has several advantages as it is relatively simple and efficient to use. It permits the isolation of total nucleic acids from a single tissue or cell preparation. The mRNA is isolated in a biologically active form, coding for high molecular weight proteins and is suitable for cDNA cloning. The DNA isolated from cells is of high molecular weight and can be subjected to restriction and Southern analysis. DNA isolated from both cells and tissues may also be used for dot blot analysis.

Acknowledgements

This work was supported by a grant to G.H. Dixon from the Medical Research Council of Canada. S.A.K. is and J.C.S. was supported by an Alberta Heritage Foundation for Medical Research post-doctoral fellowship. The authors would like to thank Mr. R.J. Winkfein for the electrophoretic analysis of the HeLa cell RNA and Ms. M. Cudmore for her expert secretarial assistance.

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